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Dominant Lethal Studies with the Halogenated Olefins Vinyl Chloride and Vinylidene Dichloride in Male CD-1 Mice

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The mutagenic activity of vinyl chloride (VC) and vinylidene dichloride (VDC) at three exposure levels was assessed in fertile male CD-1 mice with the dominant lethal test. Each compound was assessed in a separate study.

Male mice were exposed by inhalation to VC at 3000, 10,000, and 30,000 ppm and to VDC at 10, 30, and 50 ppm for 6 hr/day for 5 days. By comparison with control males exposed to air, no mutagenic effects on any maturation stage of spermatogenesis in treated males were detected. There was no significant increase in the number of postimplantational early fetal deaths as shown by the number of females with one or more early deaths or the number of early deaths/pregnancy or the number of early deaths/total implants/pregnancy. There was no evidence of pre-implantational egg losses as indicated by the total implants/pregnant female. There was also no reduction in fertility. (The reduction in fertility at 50 ppm VDC was unproven).

The lack of effect was not due to the insensitivity of the system used, since both the VC and VDC study a mutagenic effect was clearly demonstrated in male mice dosed IP with the positive control compounds cyclophosphamide (CTX) and/or ethylmethane sulfonate (EMS). During dosing these animals were housed under similar exposure conditions to those animals exposed to the test substances but with a flow of air through the exposure chambers.

Thus, neither VC nor VDC is mutagenic in the mouse at the stated exposure levels as measured by the dominant lethal test.

Introduction

VC used in the manufacture of poly(vinyl chloride) has been found to cause tumors in rats (*1*) and man (*2*). It has also been shown to produce chromosome breaks in exposed workers (*3–6*) and causes mutations in *Salmonella typhimurium* (*7, 8*). Another chlorinated monomer, VDC is also known to cause mutation in *Salmonella typhimurium* (*7, 8*). We, therefore, carried out dominant lethal studies to determine if there were any mutagenic effects of this type in mice after VC and VDC exposure at three levels. At the same time negative control animals exposed to air and positive control animals also exposed to air and given EMS and/or CTX were assayed.

Materials and Methods

Chemicals

VC was obtained from Air Products Ltd., Worsley, Walkden, Lancs., U.K. VDC was obtained from ICI Ltd., Mond Division, Runcorn, Cheshire, U.K. EMS was obtained from Koch-Light Ltd., Colnbrook, Bucks, U.K., and CTX (Endoxana) from Ward Blenkinsop Ltd., London, U.K.

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Animals

CD-1 mice (Charles River, Manston, Kent) were used throughout the experiment. Undosed females were 8–10 weeks old when mated and male mice immediately after dosing were 10–12 weeks old. Males were caged individually and females in pairs. They received food and water *ad libitum*.

Dosing of Male Mice

Dose levels for VC and VDC were selected on the basis of preliminary toxicity studies. A dose of 30,000 ppm of VC was found to be in the toxic range and this was chosen as the highest exposure level since it was desirable that the maximum tolerated dose or higher should be used. Other levels of 10,000 and 3000 ppm were also used. The required concentrations of VC were generated by mixing known volumes of VC and compressed air using rotameters as indicators. VDC was much more toxic to the mice. Exposure levels of 50, 30, and 10 ppm were selected. The required concentrations of VDC were generated by a controlled fluid-feed/atomizer technique (*9*). The method involves continuously passing a known volume of the compound through a concentric jet atomizer, where it is vaporized by a calculated volume of dry clean air. The volume of air required as calculated from Eq. (1).

$$\text{Air flow (l./min)} = \frac{\text{Syringe size (ml/cm)} \times \text{density (g/ml)} \times 24 \times 10^6}{\text{Injection rate (min/cm)} \times \text{ppm} \times \text{mol. wt.}} \quad (1)$$

During dosing the mice were housed individually in chambers made of stainless steel and glass with an internal capacity of three litres.

Most of the negative control animals and all animals dosed with CTX or EMS were housed under identical conditions during the dosing period but with a flow of air through the chambers. Those dosed with CTX were injected IP on day 5 of exposure and those dosed with EMS were given an oral dose by gavage for 5 consecutive days. Both of these substances were prepared as aqueous solutions immediately before use. Some of the animals in the negative control group controlling the VDC study were housed under normal conditions. Before test mating began, groups of mice were treated as shown in Table 1.

In the VDC study, in order to obtain a group with sufficient animal numbers some of the animals which were infertile in week 0 were included in group 4. Of the 8 survivors, 5 did not mate in the fertility study and one had a higher than average number of early deaths. However some of these males mated in the main study. One male surviving 50 ppm for 2 days died in week 2 of the study. Of the 17 animals, three in the CTX treatment group also did not mate in the fertility study. Fifty animals were included in the negative control group to provide a better data base against which to compare the treated groups. There were no statistical differences between males exposed to air and housed under normal conditions.

Mating

Fertility Testing. Male mice were caged with virgin female mice, 1 male and 2 female mice in

Table 1. Treatment groups.

Group	No. survivors/ no. animals treated (mice)	Treatment
VC Study		
Group 1	20/20	Air (negative control)
Group 2	18/20	3000 ppm VC, 6 hr/day, 5 days
Group 3	19/20	10,000 ppm VC, 6 hr/day, 5 days
Group 4	9/20	30,000 ppm VC, 6 hr/day, 5 days
Group 5	15/15	200 mg CTX in water/kg body weight, once by IP injection on day 5.
Group 6	25/25	200 mg EMS in water/kg body weight orally, once/day, 5 days
VDC Study		
Group 1	50/50	15 air, 35 normal conditions (negative control)
Group 2	20/20	10 ppm VDC, 6 hr/day, 5 days
Group 3	18/20	30 ppm VDC, 6 hr/day, 5 days
Group 4	6/20	50 ppm VDC, 6 hr/day, 2 days
	2/20	75 ppm VDC, 6 hr/day for 1 day, then 50 ppm VDC, 6 hr/day, for 1 day
Group 5	17/17	200 mg CTX in water/kg body weight, once by IP injection on day 5

each cage. After 5 days the females were transferred to other cages. The female mice were killed 15 days after first introducing them to the males and examined for pregnancies. The 106 males which survived dosing in the VC study and 113 males in the VDC study and were successful in fertilizing at least 1 female in their cage were selected for continuation in the experiment.

Experimental Mating. Two virgin female mice 8–10 weeks old were put into each of the 106 and 113 cages in which the males were individually housed. After 5 days the females were removed and rehoused in pairs. A week after the initial introduction the males were caged with another two virgin females and again left for 5 days. This process was repeated until the treated male mice had been mated at weekly intervals for 8 weeks with virgin females. The males were then killed and not examined further. No attempts were made to establish whether or when mating had occurred. Instead it was assumed that most matings leading to fertilization would occur 2 or 3 days after introducing female mice to the cages containing males.

Female mice were killed 13 days after the assumed date of fertilization, i.e., 15 or 16 days after caging females with males.

Assessment

Uteri of killed mice were examined for live implantations, early deaths, and late deaths.

Statistics

The data have been statistically analyzed as reported previously (*10*).

Results

Mating weeks after treatment are represented in Tables 2–13 by numbers 1-8 and the mating week before treatment is represented by week 0. Assessment of females which became pregnant during the fertility test yielded the data for week 0 but only data from those animals that survived treatment have been included in weeks 0-8. Tables 2–7 relate to the VC study and Tables 8–13 relate to the VDC study.

Table 2. Number and percentage of male mice which survived treatment successfully mating at each week.

Week	Group 1, air (negative control)		Group 2, VC, 3,000 ppm (6 hr × 5)		Group 3, VC, 10,000 ppm (6 hr × 5)		Group 4, VC, 30,000 ppm (6 hr × 5)		Group 5, CTX, 200 mg/kg IP		Group 6, EMS, 200 mg/kg (oral) × 5	
	No. of mice	%	No. of mice	%	No. of mice	%	No. of mice	%	No. of mice	%	No. of mice	%
0	20	100	18	100	19	100	9	100	15	100	25	100
1	20	100	18	100	18	100	7	77.8	14	93.3	3[a]	12.2
2	20	100	18	100	19	100	9	100	14	93.3	23	92.0
3	20	100	18	100	19	100	9	100	14	93.3	23	92.0
4	20	100	18	100	19	100	9	100	15	100	25	100
5	19	95.0	18	100	19	100	7	77.8	15	100	25	100
6	20	100	18	100	19	100	9	100	14	100	24	100
7	20	100	18	100	19	100	9	100	14	93.3	25	100
8	20	100	18	100	19	100	8	88.9	13	92.9	25	100

[a] $p < 0.001$.

Table 3. Number of mated females becoming pregnant.

Week	Group 1, air (negative control)		Group 2, VC, 3,000 ppm (6 hr × 5)		Group 3, VC, 10,000 ppm (6 hr × 5)		Group 4, VC, 30,000 ppm (6 hr × 5)		Group 5, CTX, 200 mg/kg IP		Group 6, EMS, 200 mg/kg (oral) × 5	
	No. pregnant	No. mated	No. pregnant	No. mated	No. pregnant	No. mated	No. pregnant	No. mated	No. pregnant	No. mated	No. pregnant	No. mated
0	35	40	30	36	28	38	15	18	25	30	37	50
1	35	40	33	36	32	36	11	18	26	30	4[a]	50
2	36	40	36	36	33	38	15	18	25	30	31[b]	50
3	34	40	30	36	34	38	16	18	26	30	40	50
4	38	40	34	36	33	38	17	18	30	30	46	50
5	35	40	34	36	36	38	12	18	28	30	47	50
6	36	40	33	36	36	38	18	18	26	28	47	48
7	37	40	31	36	37	38	18	18	22	30	41	50
8	37	40	32	36	31	37	14	18	23	28	45	50

[a] $p < 0.001$.
[b] $p < 0.01$.

Table 4. Mean total implants per pregnant female.

Week	Group 1, air (negative control)	Group 2, VC 3,000 ppm (6 hr × 5)	Group 3, VC, 10,000 ppm (6 hr × 5)	Group 4, VC, 30,000 ppm (6 hr × 5)	Group 5, CTX, 200 mg/kg IP	Group 6, EMS, 200 mg/kg (oral) × 5
0	11.9	12.6	12.0	13.0	11.3	11.8
1	12.9	13.6	13.4	16.1	8.8[a]	4.0[a]
2	12.8	12.7	13.0	13.7	10.9	8.4[a]
3	12.5	12.5	12.4	11.2	10.9	12.5
4	12.4	12.1	13.7	10.0[b]	12.6	12.7
5	13.7	12.7	14.1	12.8	12.3	12.7
6	11.7	11.9	12.5	12.6	12.5	12.1
7	11.8	11.6	11.8	12.0	11.5	12.4
8	12.7	12.1	12.6	12.1	12.5	12.5

[a] $p < 0.01$.
[b] $p < 0.05$.

Table 5. Number of pregnant females with one or more early deaths (ED).

Week	Group 1, air (negative control)		Group 2, VC, 3,000 ppm (6 hr × 5)		Group 3, VC, 10,000 ppm (6 hr × 5)		Group 4, VC, 30,000 ppm (6 hr × 5)		Group 5, CTX, 200 mg/kg IP		Group 6, EMS, 200 mg/kg (oral) × 5	
	0	≥ 1 ED	0	≥ 1 ED	0	≥ 1 ED	0	≥ 1 ED	0	≥ 1 ED	0	≥ 1 ED
0	18	17	21	9	16	22	10	5	22	3[a]	21	26
1	15	20	16	17	10	21	6	5	1	25[a]	1	3
2	19	17	18	18	13	20	6	9	2	23[a]	5	26[a]
3	18	16	16	14	19	15	9	6	8	18	15	25
4	16	22	17	17	20	13	9	7	14	16	19	27
5	17	18	18	16	14	22	3	9	12	16	25	22
6	17	19	11	22	21	15	7	11	13	13	24	23
7	18	19	13	18	21	16	8	10	10	12	22	19
8	13	24	14	18	15	16	8	6	13	10	22	23

[a] $p < 0.01$.

Table 6. Mean number of early deaths per pregnancy.

Week	Group 1, air (negative control)	Group 2, VC, 3,000 ppm (6 hr × 5)	Group 3, VC, 10,000 ppm (6 hr × 5)	Group 4, VC, 30,000 ppm (6 hr × 5)	Group 5, CTX, 200 mg/kg IP	Group 6, EMS, 200 mg/kg (oral) × 5
0	0.77	0.33	0.54	0.40	0.16	0.59
1	0.86	0.91	1.00	0.45	4.27[a]	3.50[a]
2	0.83	1.00	1.15	0.93	4.84[a]	2.58[a]
3	0.91	0.73	0.88	0.93	1.54	1.45
4	0.89	0.79	0.61	0.69	1.13	0.85
5	1.06	0.71	1.03	1.00	1.11	0.72
6	0.97	0.91	0.75	1.39	0.65	0.85
7	0.76	0.87	0.68	0.89	0.82	0.63
8	1.03	0.88	1.00	0.71	0.70	0.73

[a] $p < 0.01$.

Table 7. Early deaths as a percentage of total implants per pregnant female.

Week	Group 1, air (negative control)	Group 2, VC, 3,000 ppm (6 hr × 5)	Group 3, VC, 10,000 ppm (6 hr × 5)	Group 4, VC, 30,000 ppm (6 hr × 5)	Group 5, CTX, 200 mg/kg IP	Group 6, EMS, 200 mg/kg (oral) × 5
0	6.5	2.7	4.4	3.1	1.4[a]	5.1
1	6.6	6.7	7.5	2.7	49.1[b]	77.78[b]
2	6.4	7.9	9.6	6.9	44.6[b]	31.5[b]
3	7.3	5.9	7.0	7.8	14.1	11.6
4	7.2	6.5	4.5	6.5	9.0	6.7
5	7.8	5.5	7.3	7.9	9.1	5.7
6	8.2	7.6	6.0	11.1	5.2	7.0
7	6.4	7.3	5.7	7.4	7.0	5.1
8	8.0	7.2	8.1	5.9	5.7	5.9

[a] $p < 0.05$.
[b] $p < 0.01$.

Table 8. Number and percentage of male mice which survived treatment successfully: mating at each week.

Week	Group 1 (pooled negative control)		Group 2, VDC, 10 ppm (6 hr × 5)		Group 3, VDC, 30 ppm (6 hr × 5)		Group 4, VDC, 50 ppm (6 hr × 5)		Group 5, CTX, 200 mg/kg IP	
	No. of mice	%	No. of mice	%	No. of mice	%	No. of mice	%	No. of mice	%
0	50	100	20	100	18	100	8[a]	38	17[b]	76
1	50	98	20	95	18	100	8[a]	75	17[b]	65
2	50	96	20	100	18	94	7[b]	86	16	94
3	50	94	20	100	18	89	7[a]	43	17	94
4	49	98	20	100	18	89	7[a]	43	15	100
5	47	94	20	100	17	82	7[a]	57	17	100
6	49	88	20	100	17	100	7[a]	43	17	88
7	49	92	20	90	17	100	6[c]	50	16[c]	75
8	48	92	19	95	17	88	7[c]	57	19	86

[a] $p < 0.001$.
[b] $p < 0.01$.
[c] $p < 0.05$.

Table 9. Number of mated females becoming pregnant.

Week	Group 1 (pooled negative control)		Group 2, VDC, 10 ppm (6 hr × 5)		Group 3, VDC, 30 ppm (6 hr × 5)		Group 4, VDC, 50 ppm (6 hr × 5)		Group 5, CTX, 200 mg/kg IP	
	No. pregnant	No. mated	No. pregnant	No. mated	No. pregnant	No. mated	No. pregnant	No. mated	No. pregnant	No. mated
0	73	100	30	40	25	36	5[a]	16	17[b]	34
1	82	100	32	40	33	36	7[a]	16	20[b]	34
2	75	100	36	40	32	36	7[a]	14	25	33
3	82	100	34	40	26	36	5[a]	14	28	34
4	85	98	36	40	27	36	6[b]	14	26	32
5	80	94	34	40	26	34	6[a]	14	29	34
6	75	98	33	40	32	34	5[b]	14	25	34
7	77	98	33	40	28	34	7	13	19[c]	33
8	73	96	28	39	26	34	7	14	28	39

[a] $p < 0.01$.
[b] $p < 0.001$.
[c] $p < 0.05$.

Table 10. Mean total implants per pregnant female.

Week	Group 1 pooled (negative control)	Group 2, VDC, 10 ppm (6 hr × 5)	Group 3, VDC, 30 ppm (6 hr × 5)	Group 4, VDC, 50 ppm (6 hr × 5)	Group 5, CTX, 200 mg/kg IP
0	12.2	11.0[a]	12.0	11.4	11.5
1	12.3	13.1	12.8	11.7	9.2[b]
2	12.3	11.7	12.1	11.0	9.5[b]
3	12.0	12.1	13.2	13.4	11.0[c]
4	12.0	12.1	13.0	12.7	12.1
5	11.8	11.7	12.5	10.7	12.0
6	12.5	12.4	12.1	12.6	11.1[a]
7	12.6	13.0	12.5	12.7	11.7[c]
8	12.3	13.6	12.4	12.6	11.6

[a] $p < 0.01$.
[b] $p < 0.001$.
[c] $p < 0.05$.

Table 11. Number of pregnant females with one or more early deaths (ED).

Week	Group 1 (pooled negative control)		Group 2, VDC, 10 ppm (6 hr × 5)		Group 3, VDC, 30 ppm (6 hr × 5)		Group 4, VDC, 50 ppm (6 hr × 5)		Group 5, CTX, 200 mg/kg IP	
	0	≥ 1 ED	0	≥ 1 ED	0	≥ 1 ED	0	≥ 1 ED	0	≥ 1 ED
0	40	33	14	16	10	15	1	4	9	8
1	41	41	18	14	19	14	6	1	0[a]	20
2	39	36	18	18	17	15	3	4	2[a]	23
3	45	37	18	16	15	11	4	1	7[b]	21
4	40	45	17	19	11	16	2	4	12	14
5	41	39	15	19	11	15	3	3	12	17
6	40	35	14	19	15	17	2	3	12	13
7	43	34	15	18	15	13	5	2	10	9
8	37	36	13	15	13	13	3	4	15	5

[a] $p < 0.001$.
[b] $p < 0.01$.

Table 12. Mean number of early deaths per pregnancy.

Week	Group 1 (pooled negative control)	Group 2, VDC, 10 ppm (6 hr × 5)	Group 3, VDC, 30 ppm (6 hr × 5)	Group 4, VDC, 50 ppm (6 hr × 5)	Group 5, CTX, 200 mg/kg IP
0	0.59	0.80	0.84	2.00	1.06
1	0.72	0.72	0.61	0.29	4.00[a]
2	0.69	0.75	0.56	0.71	4.04[a]
3	0.76	0.68	0.46	0.80	2.32[a]
4	0.79	0.83	1.00	1.00	0.85
5	0.78	0.68	0.73	0.67	0.86
6	0.88	0.91	0.94	1.00	0.64
7	0.65	0.85	0.57	0.57	0.74
8	0.85	0.75	0.85	0.71	0.30

[a] $p < 0.001$.

Table 13. Early deaths as a percentage of total implants per pregnant female.

Week	Group 1 (pooled negative control)	Group 2, VDC, 10 ppm (6 hr × 5)	Group 3, VDC, 30 ppm (6 hr × 5)	Group 4, VDC, 50 ppm (6 hr × 5)	Group 5, CTX, 200 mg/kg IP
0	4.8	10.1	7.3	17.3	9.1
1	5.7	5.3	5.1	2.2	45.6[a]
2	5.5	6.9	4.4	5.7	41.4[a]
3	6.4	6.3	3.6	5.3	21.8[a]
4	6.9	7.1	7.4	10.3	6.9
5	6.6	6.1	6.2	5.6	6.9
6	7.0	7.6	8.3	7.5	5.6
7	4.9	6.6	4.5	4.3	6.1
8	7.0	5.4	7.1	5.8	2.4

[a] $p < 0.001$.

Fertility

Successful Mating Frequency. The numbers of males successfully mating at each week are shown in Tables 2 and 8. Numbers remained high during the experiments. No statistically significant differences in the mating frequency were found between VC treatment groups and the control at any week by using a chi-squared test. There was, however, a significant difference between the EMS-treated group and the negative control group in week 1. In the VDC study, the mating frequency was high in the two groups exposed to the lowest doses of VDC in all weeks by comparison with the negative control group. The mating frequency, however, was statistically significantly lower in the high exposure group in weeks 0–8 and the positive control group in the weeks 0, 1, and 7. This effect in the VDC highest exposure group was, however, probably due to infertility of the males used.

Pregnancy Frequency. The numbers of females in each group which became pregnant at each week of mating are shown in Tables 3 and 9. In the VC study, statistical differences between treated groups and the negative control group were found only in the EMS-treated group at weeks 1 and

2 by using a chi-square test. In the VDC study there were significant differences in the highest VDC exposure group at weeks 0–6 and the positive control group in weeks 0, 1, and 7. Again this was probably due to infertility of the males.

These results indicate that VC at the three exposure levels and VDC at least at exposures of 10 and 30 ppm did not cause a reduction in fertility. Any reduced fertility at 50 ppm VDC was unproven.

Total Implantations

The mean total number of implants per pregnant female in each group is shown in Tables 4 and 10. The mean values were adjusted to take account of the unequal number of pregnant females per male and were compared statistically by using an analysis of variance and a *t*-test. In the VC study statistically significant differences were evident in week 1 in the CTX-treated group and weeks 1 and 2 in the EMS-treated group. A significant difference ($p < 0.05$) in week 4 was also found between the group exposed to the highest dose of VC (Group 4) and the negative control group. In the VDC study the CTX positive control group was statistically significantly different from the negative control groups in weeks 1, 2, 3, 6, and 7. Only the VDC group to be exposed to 10 ppm showed a significant difference from the negative control group in the pre-experimental nontreatment week.

Thus there was no indication of a preimplantation loss of eggs in either study except in week 4 after 30,000 ppm of VC.

Early Deaths

The data for early deaths have been presented in various ways.

Number of Pregnant Females with One or More Early Deaths. In the VC study, CTX and EMS treatment caused increases in the number of pregnancies with early deaths (Table 5). The effect was significant (chi-square) in weeks 1 and 2 for the CTX-treated group and in week 2 for the EMS-treated group. No differences from the negative control group were seen in the VC-treated group. Similarly in the VDC study there were only significant differences in the CTX positive control group in weeks 1, 2, and 3 (Table 11).

The Mean Number of Early Deaths per Pregnancy. A large number of low or zero values were encountered, so it was necessary to stabilize the variance prior to analysis. There were no statistically significant increases in early deaths after VC treatment (Table 6). However, differences were evident in weeks 1 and 2 with CTX treatment and with EMS treatment. In the VDC study only the CTX positive control group was significantly different from the negative control group in weeks 1, 2, and 3 (Table 12).

Early Deaths as a Percentage of Total Implants per Pregnant Female. Again, it was necessary to stabilize the variance prior to analysis. In the VC study CTX and EMS treatment groups were significantly different from the negative control groups in weeks 1 and 2, whereas VC-treated groups were not (Table 7). The CTX treatment group also showed a significant difference in the week before treatment. In the VDC study only the CTX treatment group was significantly different from the negative control group in weeks 1, 2, and 3 (Table 13).

Thus with all these different methods of analysis of the data of early deaths no statistically significant differences from the negative control groups were seen in the VC or VDC treated groups.

Late Deaths

In each study late deaths were randomly distributed throughout all the groups and did not appear to be treatment-related.

Conjoined Placentae

Conjoined placentae were seen in this strain. They were the result of very close implantation sites and were not monozygotic twins (*12*). In the experiments they were classified as double implantations. Since they occurred with equal frequency in all groups they did not appear to be correlated with treatment.

Discussion

The best indication of mutagenic activity of a substance in the dominant lethal test is an increase in the number of post-implantational foetal early deaths (*13*). From the data for early deaths there was no evidence in either study of a mutagenic effect with VC or VDC at the administered exposure levels. This did not appear to be a result of lack of sensitivity of the animals used, since there was a marked response to CTX and EMS. High doses of CTX and EMS were used in our studies to obtain a highly significant positive result. However, the dominant lethal study in our hands (*14*) is at least as sensitive as that reported elsewhere. We have shown ethyl methane sulfonate on previous occasions to give a positive result with a single IP dose of 150 mg/kg body weight, which is comparable to that reported previously (*15*) for the same mouse strain.

For reasons described earlier (*10, 11*), different evaluation methods were used for the early deaths' data and much of the data in the studies were subjected to various methods of statistical analysis.

Pre-implantation egg losses, while representing some of the mutagenic effect, are not as important as postimplantational losses, for they could also arise due to other than genetic factors (*13*). Pre-implantational egg losses have been studied by comparing values of total implants in females mated with treated males and those mated with control males, as suggested by Epstein (*16*), rather than counting corpora lutea. There was no pre-implantational egg loss by comparison of VC- or VDC-treated groups with the negative control group, except in the highest VC exposure group in week 4 ($p < 0.05$). When a treatment group is significantly different from a negative control group there is generally a uniform reduction in implants, whereas in week 4 after VC treatment the low result was due to a large extent to the result from one female. Without this female, mean values would not have been significantly different from the negative control group. Therefore, this result is not considered biologically significant. Yet another reason is that there was no corresponding increase in early deaths at this time.

Late deaths also are not considered as important as early deaths in the assessment of the mutagenic potential of a test substance (*13*). Late deaths were excluded from analysis to increase test sensitivity. Gropp and Kolbus (*17*) have shown, however, that trisomies in mouse foetuses cause death and elimination before term. It might be argued that sampling for late deaths at a later stage of pregnancy might lead to different results for late deaths. However, the randomness of their distribution in both studies would suggest otherwise.

There was no reduction in fertility as measured by the mating and pregnancy frequency at any week at the exposure levels of VC, suggesting no antifertility effect. The same was true for the VDC groups exposed at 10 and 30 ppm. However, the decreased fertility in the group exposed to 50 ppm was probably due to the infertility of the males which had to be used to establish a group of sufficient size to perform the experiment.

A fertility study was undertaken prior to the experiment also for reasons described earlier (*10, 11*). From these initial fertility data the background dominant lethality of all groups was determined primarily to ascertain that there were no initial differences between groups.

Mutagenic effects of vinyl chloride have been observed in laboratory tests and in exposed workers. In this study where exceptionally high doses of VC were used, which would never be encountered by workers, no mutational effect in the germ cells was observed. A possible explanation is that the active metabolites did not reach the germ cells. Similarly the lower but more toxic levels of VDC did not produce germ cells mutations.

It can be concluded, therefore, that VC and VDC do not cause dominant lethal mutations in male CD-1 mice at 3000, 10,000, and 30,000 ppm and 10, 30, and 50 ppm, respectively.

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